RESPIRATION AND CELL DIVISION IN THE EGG OF URECHIS CAUPO

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In a preceding paper (Zeuthen, 1950b) respiratory variations have been shown to accompany the later divisions in the egg of the sea urchin *Psammechinus miliaris*. In the frog egg the first three divisions are accompanied by faint, but clear-cut variations in oxygen uptake (Zeuthen, 1946). However, special conditions pertaining to large eggs in general (cf. the discussion in this paper) make it necessary to perform studies on a smaller egg similar to those on the frog egg. The present paper deals with the respiration of dividing eggs of the echiurid worm *Urechis caupo*. It is attempted to obtain accurate information about the shape of the respiration curve and to establish the time relations between respiration and stage of mitosis. Moreover, the recent communication by Tang (1948), claiming that in the sea urchin mitotic variations in respiration can be found at high but not at low temperature, has induced the present author to perform experiments on *Urechis* at widely different temperatures.

Material

The eggs of *Urechis caupo* have proved to be excellent objects for embryological and cell-physiological studies. From male and from female animals uncontaminated sperm and eggs can be drawn throughout most of the year and each animal furnishes good sexual products over a long period. The descriptive embryology of *Urechis* has been studied extensively by Newby (1940). After the addition of sperm the eggs undergo reduction divisions. These are followed by regular mitotic cell divisions, one division every 45 minutes at 15° C. Fertilization is usually almost 100 per cent and the divisions are synchronous within a few minutes in the same lot. After the 16 cell stage has been reached, the spiral cleavage of the egg becomes apparent, i.e., within the single embryo different quarters of blastomeres divide at different rates. The present study, therefore, is confined to the period in which the reduction divisions and the first 4 regular mitotic divisions take place.

Methods

The Cartesian diver method as described by Zeuthen (1950a) was used. The diver had an air volume of 0.8 μl–1.1 μl. The water volume was 2–3 times this. Thus in the experiments with O_2 in the diver 90–94 per cent of the oxygen present was in the gas phase of the diver; hence 90–94 per cent of the oxygen consumed by the eggs was manometrically measurable.

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After fertilization, 10 minutes were allowed to pass in order that it could be ascertained that the eggs were fertilized (to nearly 100 per cent). Then the diver was charged and transferred to the flotation vessel. After this about one hour elapsed before saturation equilibrium was established in the diver and reliable measurements could be obtained. Usually the mitotic divisions had not started until after this period, so that the 3 to 4 first mitoses could be studied.

The appearance of the cleavage furrows could be observed in the diver itself through the horizontal measuring microscope. Not every egg in the diver could be observed, but a representative number could always be followed. After the experiment the eggs were removed from the diver and brought under a regular microscope. The development was always as good as in the control outside the diver. In the diver the eggs lay densely packed in a layer the thickness of which, however, never exceeded 0.5 mm. Except in some few experiments at low temperature, about 400–700 eggs were employed in each experiment. As will appear below, the oxygen supply in all experiments was ample.

In the graphs the respiratory rate (expressed in cm. Brodie/min.) is plotted against the time. Each measurement is plotted in the middle of the period for which it is valid. The lines above the individual curve indicate the division periods.

Experiments

Diver without eggs, with unfertilized eggs, and with fertilized eggs

If a diver is charged in the usual way, but without respiring objects in the water, we obtain curves of the types I or II in Figure 1. In experiment I the diver contains a bubble of atmospheric air, in experiment II it contains pure oxygen. In the first part of the experiment we measure an apparent respiration, which is faint in experiment I, but very pronounced in experiment II. In both cases the apparent respiration can be explained by assuming that a saturation equilibrium is becoming established in the diver. If the diver is charged with an air bubble, there is a good chance that the fluids of the diver from the beginning are in equilibrium with the gas phase. The fact, however, that the diver will usually have become a little heated by the hand during the filling procedure may explain why, even in this case, we often measure a slight uptake of gas during the first part of the experiment (decrease in solubility of gases with increasing temperature). In experiment II (O_2 in diver) the fluids of the diver were from the beginning in an approximate equilibrium with atmospheric air. In contact with oxygen, gas is taken up because oxygen is more soluble in water than is air.

Curve III shows the results with about 700 unfertilized eggs in the diver (O_2 in diver). After the initial period of equilibration within the diver is over, we measure a respiration which is gradually increasing, the increase, however, following a very smooth curve without any indications of a rhythm. Evidently, the accuracy with which the respiration over short periods can be measured is very high, the largest deviations from the average being about 1 per cent and the error on the single measurement being very much smaller.

Curve IV shows an experiment with about 500 fertilized eggs in the diver (O_2 in diver). All eggs divided almost synchronously, and simultaneously with the eggs outside the diver. The lines numbered 1–3 above the curve indicate the division

periods as they could be observed in the diver itself. The time interval indicated by each line shows the whole division period. The bulk of the eggs, of course, divided within a distinctly narrower time interval. The respiration of the eggs fluctuates in waves: there is one respiratory wave per cell division and the cleavage period of each mitosis appears just after the respiration has passed the maximum.

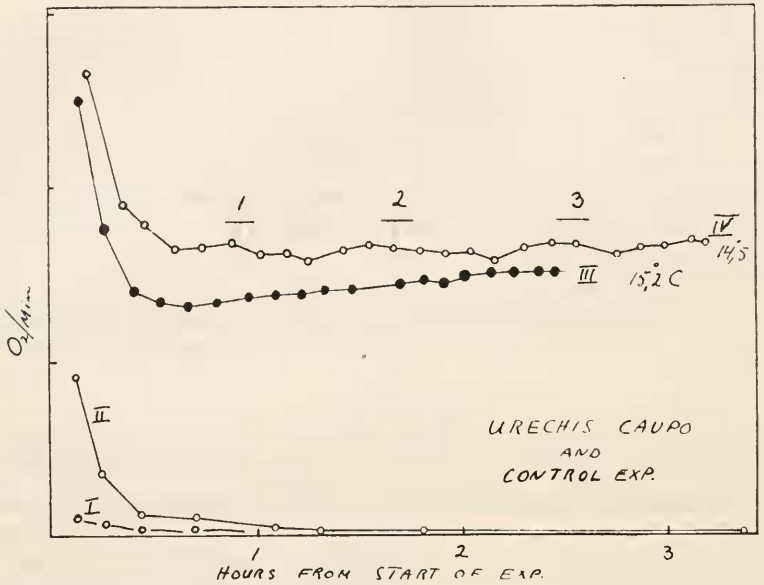

FIGURE 1. Control experiments with empty diver (I, II), experiment on unfertilized eggs (III) and on fertilized eggs (IV). 1st, 2nd, 3rd cleavages are indicated with horizontal lines.

A comparison between curves III and IV leaves little doubt that the respiratory waves are significant. Moreover, they have been found in all, e.g., about 10 experiments.

Experiments with dividing eggs at different temperatures. Oxygen in the diver

Tang (1948) considers the size of the respiratory waves accompanying cell division in the sea urchin egg to be highly influenced by temperature. The eggs of *Urechis* divide at temperatures ranging from about 9° C. to about 24° C. Above and below these temperatures cleavage becomes abnormal or fails altogether so that syncytia may develop. Experiments were carried out with normally developing eggs at 9.8–10° C., at 13–15° C. and at 22.8° C. Some of these experiments are assembled in Figure 2. The units on the ordinates have in each case been chosen so that the curves are directly comparable. The lower the temperature, the slower is the development and the less intense the respiration. Therefore, it is technically difficult to demonstrate the respiratory rhythm at low temperatures. The waves, however, are present at all temperatures. We shall return to this point in the discussion.

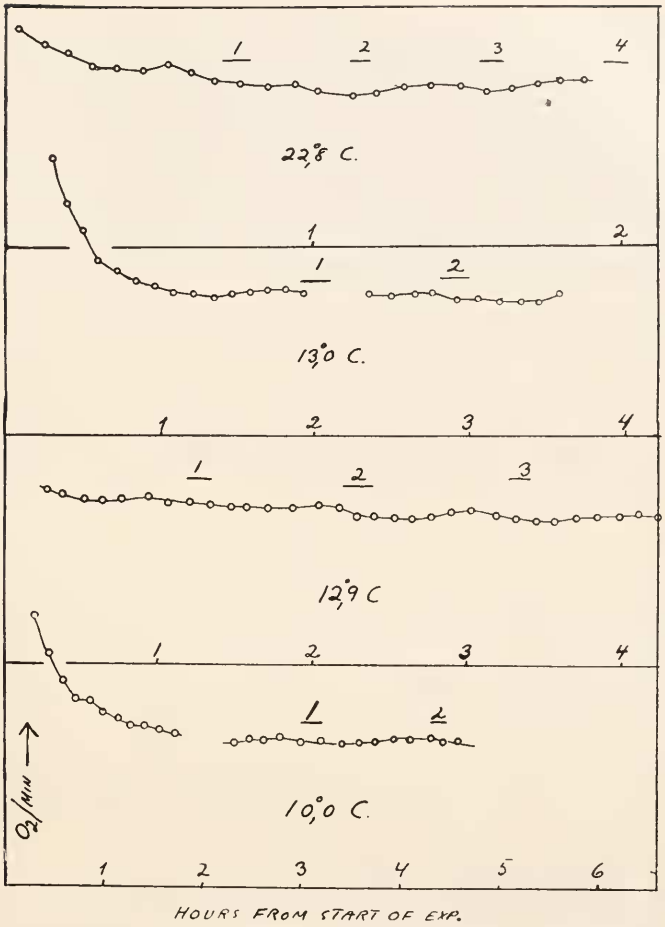

FIGURE 2. Within wide limits the respiratory waves appear to be uninfluenced by temperature.

Dividing eggs in a diver containing atmospheric air

The eggs seem to develop just as well in the diver where a high oxygen tension is prevailing as they do in the control (an open Petri-dish floating in the same thermostat as is used for the diver experiments). However, the respiratory waves might conceivably be influenced by the high oxygen tension in the diver without the cell division itself being in any way affected. The experiment, the result of which is shown in Figure 3, however, would seem to rule this possibility out. In this case the diver is filled with atmospheric air, and the eggs respire and divide normally until the 4th cleavage is completed. After this time signs of oxygen deficiency appear: respiration goes down and the cells divide more slowly than the controls outside the diver. The oxygen tension (expressed in per cent of one atm.) can, with fair accuracy, be calculated from the change in equilibrium pressure of the diver, assuming the oxygen pressure to be 20.9 per cent of an atm. at the moment when the diver is closed.

Curve II indicates oxygen pressure in the air bubble calculated in this way. The critical value seems to be about 6 to 7 per cent. This of course corresponds to even lower oxygen pressures on the respiring cells. The pressure difference between the gas phase and water which will allow enough oxygen to diffuse to the respiratory centers in this particular diver is evidently small. In most cases the divers which contained an oxygen bubble had about the same dimensions as had the diver used in the experiment of Figure 3. This would indicate that, in the oxygen experiments, the oxygen pressure in the eggs themselves was very close to one atmosphere. Thus, mitotic waves in oxygen consumption of dividing *Urechis* eggs have been demonstrated within wide limits of oxygen pressure.

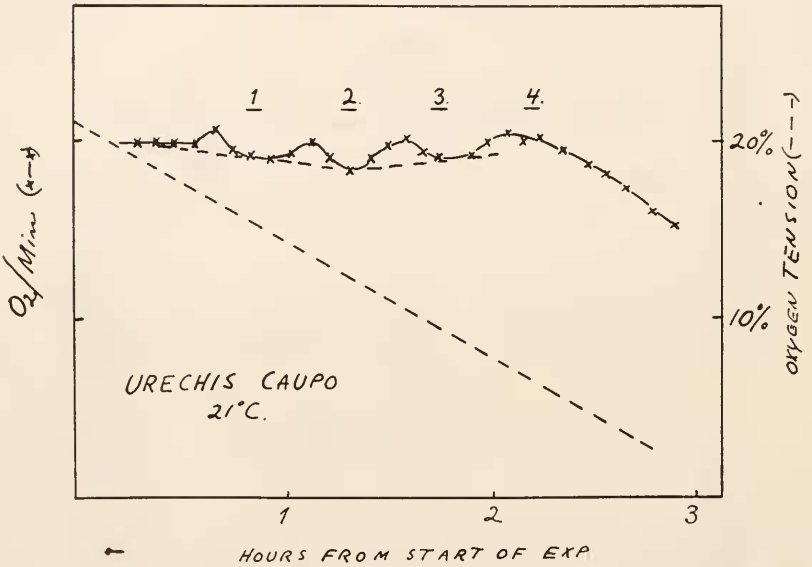

FIGURE 3. The respiratory waves can be demonstrated also at low oxygen pressures.

It may be worth while here to point out that the apparent difference between the curves of Figure 1 and Figure 2 is due only to a slight difference in the way of plotting. In Figure 1 the time scale is relatively long, and the respiratory waves therefore relatively long and flat. In Figure 2 the time scale is short and the waves shorter and steeper.

Oxygen uptake during the reduction divisions

There are some indications that the reduction divisions also result in slight changes in respiratory rate in the *Urechis* egg. In some cases the experiment was started so early that the reduction divisions occurred in the diver, though during the early part of the experiment at a time when the saturation equilibria were not yet established. However, two small waves seem to be superimposed on the curve, one wave per reduction division. Admittedly, these waves (to be seen on Fig. 2, 13°–10.0° C.) are very uncertain. Probably the safest conclusion to be drawn from these experiments is that, if the reduction divisions are accompanied by changes in the respiratory metabolisms, these changes are only slight.

Cytological observations

The time relations between stage of mitosis, cytoplasmic cleavage and fluctuations in respiration intensity were established in special experiments. Eggs from the same fertilization batch were kept in the diver and outside the diver at the same temperature and air or oxygen was bubbled through the outside suspension. Samples were drawn at regular intervals for cytological examination (Feulgen staining). The respiration was found to be low or increasing when the nuclei are in prophase. In those stages of actual mitosis when the nuclear membrane is dissolved away (meta-, ana-, and early telophase) the respiration is slightly increased. Maximum of respiration would seem to occur around metaphase-anaphase. Cytoplasmic division takes place during telophase at a time when respiration is already decreasing.[2]

The experiments indicate that the time relations between cytoplasmic cleavage and the different stages of mitosis are the same whether oxygen or atmospheric air is blown through the egg suspension. It should also be recalled that in all previous experiments the eggs cleaved at the same time in the diver (with air or with oxygen) as in the control (with air). All observations, tied together, support the general concept outlined above.

Discussion

The respiratory rhythm found in dividing *Urechis* eggs very much resembles that previously described for the frog egg (Zeuthen, 1946). Due to a series of complicating conditions, however, Linderstrøm-Lang (1946) considered that the observed respiratory waves in the frog egg, while qualitatively true, were slightly delayed and somewhat damped as compared with the events in the cells themselves. The chief difficulty was that CO_2 was not chemically absorbed but only held in solution by a large volume of water. Moreover, the diffusion distances between the air bubble and the metabolic centers in the large egg were too long.

In the present experiments advantage has been taken of Linderstrøm-Lang's calculations. In the inverted diver the respiratory CO_2 is chemically absorbed and the eggs lie on the water-air meniscus, so that the distance between the gas bubble and the most remote eggs never exceeds 0.5 mm. (exception: experiment at 9.8–10° C., see below). The damping factor, φ, of Linderstrøm-Lang is between 0.99 and 1.00, meaning that the damping of the respiratory waves is less than 1 per cent. The delay, t_D, is about 1 minute. Thus damping and delay are well within the experimental error. Changes in respiratory rate that are not too fast are picked up and recorded accurately with the instrument used.

The situation is slightly different with the experiment at 9.8–10° C. Because of the low respiration more than 1000 eggs had to be used. Therefore, the layer of eggs plus water separating the most remote eggs from the gas phase was 0.85–1.00 mm. The damping of the amplitude of the respiratory waves may in these experiments have amounted to 10 per cent, and the delay in recording the waves may have been 3–4 minutes. In view of the fact, however, that the waves are very long at this temperature, the delay is insignificant. In Table 1, a 10 per cent correction for the lower wave amplitude in these particular experiments has been applied. A comparison of the curves in Figure 2 reveals that temperature can hardly be con-

[2] The author wishes to record his sincere thanks to Dr. Irving A. Tittler, who made the microscopic preparations and studied the slides.

sidered significantly to influence the respiratory waves in the *Urechis* egg. If Tang (1948), however, considers that the respiratory waves are technically more difficult to demonstrate at a lower than at a higher temperature, we agree completely.

In Table 1 an intercomparison of all published curves is given. The wave heights are defined as described for the frog (Zeuthen, 1946). No certain dependency between the height of the respiratory waves and temperature or oxygen pressure can be observed. Figure 3 furnishes very slight evidence that the waves accompanying the later divisions may be slightly larger than those accompanying the first divisions. Figures 1 and 2, however, do not support such a view. The extra oxygen consumption of the first 3–4 divisions constitutes a very small fraction of the total respiration, e.g., 2–3 per cent only. Thus, the extra oxygen consumed per

TABLE I

	Wave heights in % of total					
	in oxygen					in air
Division no. \ Temperature	9.8°	10.0°	12.9°	13.0°	22.8°	21.0°
1	5.0	3.5	3.5	5.0	6.0	5.5
2	—	3.5	6.0	5.0	4.0	6.0
3	—	—	6.5	—	5.0	8.0

mitotic cycle is low, and probably it increases only slightly or not at all as we go from the 1st to the 3rd or 4th division. In this respect there seems to be a difference between the first divisions in *Urechis* and the later divisions (no. 5–8) in *Psammechinus*. It remains to be seen whether this is a difference between eggs or a difference between division stages, although the latter possibility appears to be the more likely one (Zeuthen, 1950b).

The physiological and biochemical function of the "extra oxygen" consumed during mitosis is unknown. However, since "mitotic variations" in oxygen uptake were found in a fertilized frog egg, which for some reason did not divide (Zeuthen, 1946), no close connection is indicated between respiratory processes and cytoplasmic cleavage. The fact that in *Urechis* (and *Psammechinus*) the cytoplasm always divides during a short period of decreasing respiration is an argument against the theories forwarded by some authors (discussed by Loeb, 1913) that the general increase in respiration which takes place while the sea urchin egg develops into a blastula correlates with the cytoplasmic cleavages and the resulting surface enlargement of the embryo. Moreover, comparing the eggs of the frog, the sea urchin and *Urechis* we find that the time relations between respiratory waves and cytoplasmic cleavage vary: In the two small eggs the cytoplasm cleaves in 2 or 3 minutes during telophase (decreasing respiration), but in the frog egg (*Rana fusca*, Brachet, 1934, 1935) the cytoplasm already starts to divide at the animal pole of the egg during prophase (increasing respiration). However, in the frog it takes a long time before the furrow embraces the whole egg. It seems probable that in all three eggs the time relations between stage of nuclear division and of respiratory changes may be

much the same as found in the egg of *Urechis*. Thus the bulk of evidence is in favor of the theory that cleavage is more or less dissociable from the two more closely correlated processes of nuclear division and fluctuations in oxygen uptake. The experiments of Runnström (1934) to be discussed below throw some light on this latter correlation.

According to Runnström (1934) the first part of the mitosis in the sea urchin egg is strictly dependent on the presence of oxygen. In an atmosphere of nitrogen or in the presence of KCN the eggs are not capable of carrying out those steps of the mitosis which lead to the dissolution of the nuclear membrane. If, however, the eggs are brought under anaerobic conditions or if they are poisoned with KCN at any stage after the anaphase (diaster), the eggs will divide synchronously with the controls in air. Unfortunately, such experiments have not been performed on *Urechis*. Provided both eggs are really comparable, it would seem that oxygen is required during the period of increasing respiration, but that it is not necessary when the respiration is again decreasing. Perhaps, therefore, an increase in respiration in the first part of the mitosis is *indispensable* for the mitotic process in the sea urchin and in *Urechis*. In the frog this is *not* so. Brachet (1935) found that this egg is capable of development to the young blastula stage in the complete absence of oxygen. Here fermentative energy evidently can take the place of oxidative energy even during the first part of the mitosis.

Zeuthen (1946) mentions the fact that in the frog egg the mitotic variations in O_2-uptake can be interpreted not only as variations in the rate of oxidations in the egg. Even if the egg respires at an absolutely uniform rate the rate of O_2-uptake from the surroundings will vary with possible changes in the permeability of the egg surface to O_2. In the latter case the "respiratory waves" measured will actually only be variations in the amount of oxygen physically dissolved in the egg. The presented accumulation of further experimental facts on other, more intensely respiring and probably more permeable eggs, however, makes a uniform interpretation on the basis of the permeability theory of all results almost impossible. There seems to be little doubt, then, that what we measure is in reality variations in the rate of oxygen utilization in the eggs.

My sincere thanks are due to Dr. L. R. Blinks and to Dr. C. B. van Niel, both of Hopkins Marine Station, for the interest they showed in the present work.

Summary

The first 3–4 mitotic divisions in the egg of the echiurid worm *Urechis caupo* were found to be accompanied by slight (2–3 per cent) increases in respiration. The phenomenon was found at all temperatures at which normal development takes place (9–24° C.), and it was found with air as well as with oxygen in the diver. The respiration was low or beginning to rise during prophase. It was maximum around metaphase-anaphase, lower during telophase when the cytoplasm divides.

In the discussion the experiments are considered in relation to previously recorded observations.

LITERATURE CITED

Brachet, J., 1934. *Arch. f. exp. Zellforsch.*, **15**: 46.
Brachet, J., 1935. *Arch. Biol.*, **46**: 1.

Linderstrøm-Lang, K., 1946. *Compt. rend. Lab. Carlsberg,* Sér. chim., **25**: 229.
Loeb, J., 1913. Artificial fertilization and parthenogenesis. Chicago.
Newby, W. W., 1940. *Mem. of the Amer. Philos. Soc.,* Philadelphia.
Runnström, J., 1934. *Protoplasm,* **20**: 1.
Tang, P. S., 1948. *Nature,* **162**: 189.
Zeuthen, E., 1950a. Cartesian diver respirometer. *Biol. Bull.,* **98**: 139.
Zeuthen, E., 1950b. Respiration during cell division in the egg of the sea urchin *Psammechinus miliaris*. *Biol. Bull.,* **98**: 144.